BAND 3-PROTEIN FROM HUMAN ERYTHROCYTE MEMBRANES STRONGLY INTERACTS WITH CHOLESTEROL

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1. Introduction

Band 3-protein [1], the main integral protein of the human erythrocyte membrane, is strongly involved in anion transport, the membrane's most prominent function (for review, see ref. [2]). Since, apparently, the biological function of an integral membrane protein is exerted by a combined action of the protein and neighbouring lipid and protein molecules (e.g., [3–5]), efforts aimed at a better understanding of the mechanism of anion transport may benefit from studies on the interactions of the band 3-protein with other components of the erythrocyte membrane. Moreover, such studies are of obvious interest for the problem of erythrocyte membrane structure. Several investigations have already been performed on the protein–protein interactions of the band 3-protein [1,6,7]. No information is available, however, on the interactions of this protein with lipids.

Recently, we have developed a new method for the isolation of the band 3-protein which avoids the use of detergents [8]. Using this method of protein preparation, we were now able to study the interactions of the protein with lipid monolayers at the air–water interface. These experiments, which are described in this paper, have revealed a strong interaction of the band 3-protein with cholesterol.

2. Materials and methods

2.1. *Materials*

The phospholipids, phosphatidylcholine (PC) and phosphatidylethanolamine (PE) from egg yolk, bovine sphingomyelin (Sph) and phosphatidylserine (PS) from bovine spinal cord, were purchased from Lipid Products, South Nutfield, Surrey, England ('grade I' purity > 99%). Cholesterol was obtained from Merck, Darmstadt, FRG ('for biochemical purposes' purity 99%). For the monolayer experiments, all lipids were dissolved in chloroform at a concentration of 1.0 mg/ml.

For the isolation of band 3-protein from human erythrocyte membranes, the lipoprotein pellet obtained after solubilization of the 'loosely bound' membrane proteins [9,10] was dissolved in 90% acetic acid and then subjected to preparative electrophoresis (in acetic acid). Details of the isolation procedure will be described elsewhere [8]. The protein was freed from acetic acid by dialysis against 0.1 mM HCl and then titrated, to pH 10.0, by addition of 0.1 M NaOH. Before being added to the monolayer trough, it was diluted to a concentration of approx. 80 μg/ml. In the analytical ultracentrifuge, the samples showed a peak with a $s_{20,w}$-value of 16, sometimes accompanied by a small peak of approx. 12 S. For some experiments, a subfraction with lower S-values was isolated [11]. The protein in these samples showed two peaks of about equal height, with $s_{20,w}$-values of 4 and 7.

2.2. *Methods*

Surface pressure changes of lipid monolayers at the air–water interface were studied in a rectangular teflon trough (5.0 × 5.0 × 2.0 cm) using a Pt Wilhelmy plate, a Beckman LM 500 electrobalance and a mV recorder, as described by Demel et al. [12].

The subphase into which the protein was injected was 100 mM NaCl, 10 mM sodium phosphate (pH 7.1). Temperature was kept at 22.0 ± 0.5°C.

The determination of protein and phospholipid concentration and analytical ultracentrifugation were performed as described earlier [9].

3. Results

When band 3-protein from human erythrocyte membranes ($s_{20,w}$ = 16) was injected underneath monolayers of different phospholipids (representing the main phospholipid classes of the erythrocyte membrane) and of cholesterol, a pronounced increase in monolayer surface pressure π was observed. The rate $d\pi/dt$ of the pressure change was large during the first minutes but later became smaller and finally vanished. The final values of π as well as the initial values of $d\pi/dt$ were strongly influenced by the chemical nature of the lipid used and were much higher for monolayers of cholesterol than for the phospholipids films (fig.1). Thus, among the lipid monolayers tested, there is a preferential interaction of the protein with monolayers of cholesterol.

The magnitude of $\Delta\pi$, the protein-induced change in monolayer surface pressure, was dependent also on the protein concentration in the subphase of the films. However, all lipids were affected in a similar way. As a consequence, monolayers of cholesterol showed the largest pressure changes at each protein concentration tested (fig.2).

Changes in the initial monolayer pressure π_i also affected the magnitudes of the pressure changes, higher values of π_i leading to lower values of $\Delta\pi$. This influence of π_i was, however, much smaller for monolayers of cholesterol than for the phospholipid films (fig.3), as was observed with some other proteins

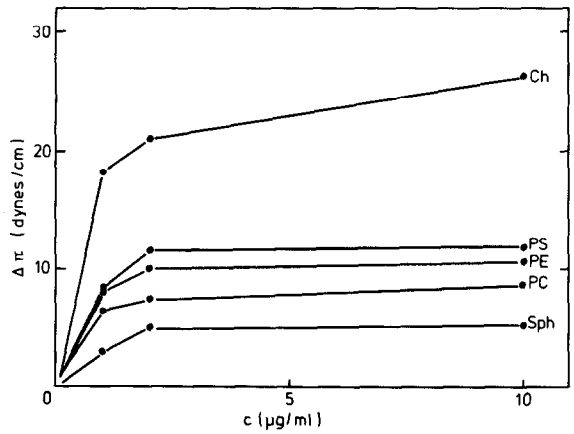

Fig.2. The effect of protein concentration c on the surface pressure increase $\Delta\pi$ induced by the protein. Initial film pressure: 10 dynes/cm.

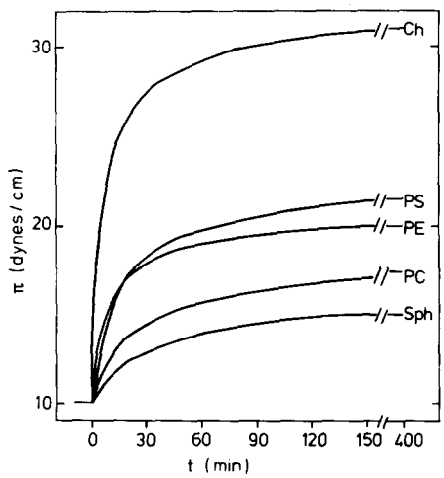

Fig.1. Changes in surface pressure π following the injection of solubilized band 3-protein (c = 2.0 μg/ml) into the subphase of different lipid monolayers. Initial film pressure: 10 dynes/cm.

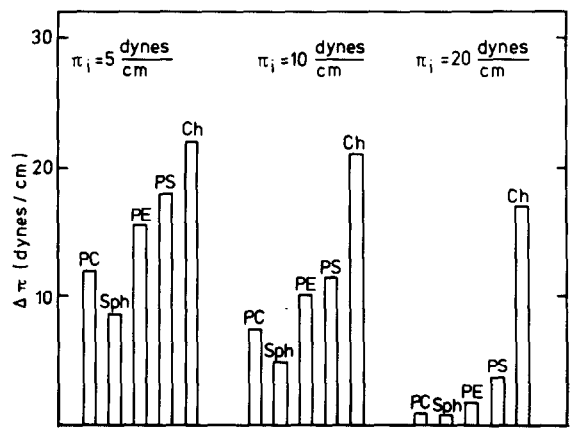

Fig.3. The effect of initial film pressure π_i on the protein-induced pressure increase $\Delta\pi$. Protein concentration: c = 2.0 μg/ml. The values of $\Delta\pi$ were taken from the plateau region of the $\pi = f(t)$-curves.

[13,14]. Thus, the preference of the protein to interact with monolayers of cholesterol, as compared to those of the phospholipids, increased with increasing π_i. At values of π_i between 25 and 36 dynes/cm, cholesterol was the only lipid still showing an interaction with the protein.

Since protein–lipid interactions may be highly influenced by the state of protein aggregation [11], some monolayer experiments were performed using protein samples with $s_{20,w}$-values of 4 and 7, instead of $s_{20,w} = 16$. However, the results obtained did not differ significantly from those described above.

4. Discussion

As shown above, the interactions of the isolated band 3-protein from human erythrocyte membranes with monolayers of cholesterol are much stronger than with phospholipid monolayers. This is demonstrated both by the magnitudes of the protein-induced changes in monolayer surface pressure and by the time course of the pressure changes. This may indicate a higher affinity of the protein for the sterol, as compared to the phospholipids; there may, however, be other reasons [8]. On the other hand, the changes in surface pressure for monolayers of cholesterol are large also on an absolute scale: they are among the largest observed with all lipid monolayer–protein systems described up to now, at least as far as studies using physiological conditions of pH and ionic strength are concerned. In our opinion, it is very unlikely that the occurrence of such a strong protein–lipid interaction in a monolayer experiment could be without biological significance. We therefore conclude that there are strong interactions between the band 3-protein and cholesterol also in the native erythrocyte membrane, cholesterol thus being an important component of the microenvironment of the protein.

A few demonstrations or at least indications of the existence of protein–cholesterol interactions in biological membranes have already been reported [15–17]. For the erythrocyte membrane, the occurrence of such interactions may be inferred from the susceptibility of several protein-mediated transport processes, including those of certain organic anions, to artificial or dietary changes in the membrane's cholesterol content [2]. The experiments described in this paper are the first direct demonstration of a strong interaction between cholesterol and a main protein component of the erythrocyte membrane In addition, they may be a starting point for studies on the structural basis of these interactions.

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